APPLIED AND ENVIRONMENTAL MICROBIOLOGY, Dec. 1987, p. 2922–2928
0099-2240/87/122922-07$02.00/0

Vol. 53, No. 12

Comparison of Four Membrane Filter Methods for Fecal Coliform Enumeration in Tropical Waters

JESÚS SANTIAGO-MERCADO AND TERRY C. HAZEN†*

Microbial Ecology Laboratory, Department of Biology, College of Natural Sciences, University of Puerto Rico, Rio Piedras, Puerto Rico 00931

Received 15 July 1987/Accepted 22 September 1987

Four membrane filter methods for the enumeration of fecal coliforms were compared for accuracy, specificity, and recovery. Water samples were taken several times from 13 marine, 1 estuarine, and 4 freshwater sites around Puerto Rico, from pristine waters and waters receiving treated and untreated sewage and effluent from a tuna cannery and a rum distillery. Differences of 1 to 3 orders of magnitude in the levels of fecal coliforms were observed in some samples by different recovery techniques. Marine water samples gave poorer results, in terms of specificity, selectivity, and comparability, than freshwater samples for all four fecal coliform methods used. The method using Difco m-FC agar with a resuscitation step gave the best overall results; however, even this method gave higher false-positive error, higher undetected-target error, lower selectivity, and higher recovery of nontarget organisms than the method using MacConkey membrane broth, the worst method for temperate waters. All methods tested were unacceptable for the enumeration of fecal coliforms in tropical fresh and marine waters. Thus, considering the high densities of fecal coliforms observed at most sites in Puerto Rico by all these methods, it would seem that these density estimates are, in many cases, grossly overestimating the degree of recent fecal contamination. Since *Escherichia coli* appears to be a normal inhabitant of tropical waters, fecal contamination may be indicated when none is present. Using fecal coliforms as an indicator is grossly inadequate for the detection of recent human fecal contamination and associated pathogens in both marine and fresh tropical waters.

The method using Difco m-FC agar for fecal coliform recovery was first included in *Standard Methods for the Examination of Water and Wastewater* (1) in 1971. Since then, several studies have suggested that this technique works better than total coliform methods for assaying the microbiological contamination level of different types of water, e.g., potable and recreational (4, 5, 7, 8, 20). Fecal coliform methods are more specific and less ambiguous than total coliform methods (4, 5, 7, 8, 20). However, it should be noted that the drinking water regulations in most countries are still based upon total coliform assays. Several m-FC methods have also been developed to improve recovery by providing for the resuscitation of physiologically injured cells and increasing the specificity of the method towards the target organism, *Escherichia coli*.

Nowhere is the importance of accurate determination of recent human fecal contamination greater than in the tropics. The diversity and severity of waterborne diseases is greatest in tropical environments. Since most countries in tropical climates are underdeveloped, with poor medical services, and large populations that are undernourished and ill housed, waterborne diseases may have a much greater effect on public health in the tropics than in temperate areas. Few studies have examined the efficacy of total coliform and fecal coliform standards in the tropics. Lavoie (20) compared isolates from fecal coliform and total coliform assays of well water in the Ivory Coast and found a much higher proportion of *E. coli* isolates from m-FC agar. Although Lavoie (20) found high densities of fecal coliforms (51 CFU/100 ml) in his untreated groundwater samples, he assumed this reflected a high degree of fecal contamination of his samples. Our own studies in Puerto Rico have shown that *E. coli* is capable of surviving for long periods of time in tropical rivers and that it can be isolated from many habitats not known to have any human or animal fecal contamination (6, 16, 21). In addition, we have also found that *E. coli* can survive even in certain polluted marine environments (9, 16, 22, 27). Fujioka et al. (10) have also shown that *E. coli* may be a normal inhabitant of fresh waters in Hawaii. These studies suggest that *E. coli* may not be a suitable indicator of fecal contamination in tropical waters. Since the target organism in m-FC assays is *E. coli*, m-FC assays may not be appropriate for tropical waters.

Pagel et al. (25) comprehensively examined four membrane filter methods for fecal coliform enumeration in various waters in Canada. We used the same methods for fecal coliform enumeration of waters in Puerto Rico to determine how these tests performed in tropical waters and to compare their performance with that found by Pagel et al. (25) in temperate waters.

(This research was conducted by J. Santiago-Mercado in partial fulfillment of the requirements for the M.S. degree from University of Puerto Rico, Rio Piedras, 1986.)

MATERIALS AND METHODS

Study sites. Eighteen sampling sites (13 marine, 4 freshwater, and 1 estuary) were chosen from six different sampling areas for their accessibility and diversity of water quality (Fig. 1). Four sites along San Juan beaches (area 2) which receive the input of storm drains and illegal sewage discharge from the city of San Juan were sampled. This area constitutes the major hotel and tourist zone. Two sites were sampled from Mata de la Gata (area 4), a mangrove picnic island in the municipality of Lajas, previously described by López-Torres et al. (21). Mata de la Gata island is adminis-

* Corresponding author.
† Present address: Environmental Sciences Division, Savannah River Laboratory, E. I. du Pont de Nemours & Co., Inc., Aiken, SC 29808.

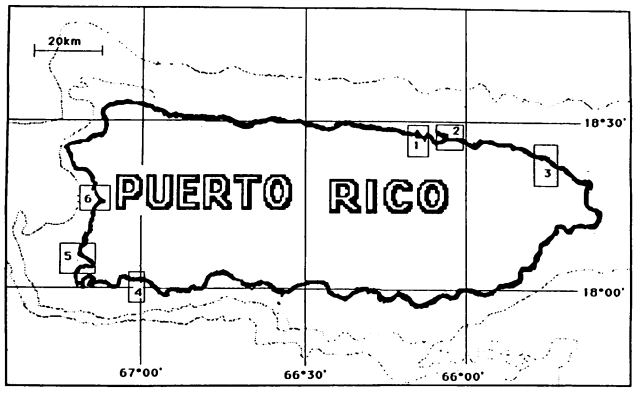

FIG. 1. Map of sampling sites in Puerto Rico.

stered by the Department of Natural Resources of the Commonwealth of Puerto Rico, and its waters receive the sewage from two latrines. Two sites were sampled in Boqueron (area 5), one in a lagoon which receives effluent from two primary sewage treatment plants and which is also the primary mangrove oyster harvesting waters for Puerto Rico, and an adjacent beach site facing the Caribbean Sea. Two sites were sampled in Mayagüez Bay (area 6), the second largest port of Puerto Rico; one site receives effluent from a tuna fish cannery, and the other site receives sewage effluents. Three sites sampled in Boca Vieja Cove (area 1), detailed previously by Biamón and Hazen (3), received 635,000 gal (2,403,475 liters) day^{-1} of untreated effluent from the largest rum distillery in the world. The Mameyes River watershed (area 3) sampling sites included the pristine upper third, near its origin at an elevation of 1,000 m in the Luquillo Experimental Forest, as well as sites downstream, which receive domestic sewage and drain into the Atlantic Ocean (for details, see Carrillo et al. [6]).

Water quality. Seven water quality parameters were measured simultaneously with water collection for bacterial densities. Measurements were taken in situ for conductivity, salinity, pH, dissolved oxygen, light intensity, and temperature. The pH, dissolved oxygen, conductivity, and temperature were measured with a Hydrolab Surveyor digital model 4041 (Hydrolab Corp., Austin, Tex.). A model 33 S-C-T meter (Yellow Springs Instrument Co., Yellow Springs, Ohio) was used to measure salinity. Turbidity, alkalinity, hardness, and ammonia measurements were done in the field with a Mini Spectronic 20 spectrophotometer (Bausch & Lomb, Inc., Rochester, N.Y.). Three liters of water was collected and divided into various bottles, and small amounts of the following preservatives were added: sulfuric acid, zinc acetate, and mercuric chloride. Amber bottles were used for samples to be analyzed for chlorophyll. All samples were then placed on ice for transport to the laboratory and analyzed within 6 h. The preserved samples were analyzed for the following parameters: nitrates plus nitrites, sulfates, inorganic phosphates, total phosphorus, and chlorophyll *a* by standard methods described previously (1).

Sampling protocols. Several grab samples were taken from each site over an 18-month period using Whirl-Pak bags (Nasco, Ft. Wilkinson, Wis.). If the sample contained chlorine, samples were collected in Whirl-Pak bags containing sodium thiosulfate (Nasco).

Media and enumeration techniques. Accuracy tests and positive controls were done with *E. coli* 104 and *E. coli* B (ATCC 10798 and ATCC 23848, respectively). Cultures were maintained on brain heart infusion agar (BHIA; Difco Laboratories, Detroit, Mich.) at 20°C under paraffin oil and subcultured every 3 months.

Appropriate volumes (allowing recovery of 10 to 100 colonies per filter), with a minimum of 1 ml, were filtered through 47-mm-diameter, 0.45-μm-pore-size, gridded, type HA, membrane filters (Millipore Corp., Bedford, Mass.) by the standard m-FC technique described previously (1). Filters were then placed on one of the following media: MacConkey membrane broth (MMB), mTEC (7), m-FC agar (Difco), and m-FC agar with a 2-h resuscitation step (m-FC2). Plates of MMB were incubated at 44.5°C for 20 ± 1 h, after which all yellow, yellow-green, and yellow-brown colonies were counted as fecal coliforms. Plates of mTEC agar were incubated at 35.0°C for 2 h, followed by incubation for 23 ± 1 h at 44.5°C, and all yellow, yellow-green, and yellow-brown colonies were counted as fecal coliforms. To gain further information in specificity testing, filters were transferred from mTEC agar to a urea substrate, and any positive colonies which were urease negative (yellow) were considered *E. coli*. Rosolic acid was omitted from the preparation of m-FC agar for both the standard (one-step) and resuscitation (two-step) methods. Incubation of m-FC agar was at 44.5°C for 24 ± 1 h, whereas in the m-FC2 method, the plates were incubated under the same condition as in the mTEC procedure. All blue or partially blue colonies were counted in both m-FC methods as fecal coliforms. For all methods, plates were incubated in tight-fitting petri dishes in a humid, block-type FC incubator (Millipore). All techniques, methods, and media were as described by Pagel et al. (25). All incubation conditions were as described previously (1). Fecal coliform densities were also determined by the MPN technique, with A-1 broth, EC medium, and brilliant green lactose bile broth as previously described (1). Identification of isolates was confirmed with API-20E (Analytab Products, Plainview, N.Y.) and tube-type indole–methyl red–Voges-Proskauer–citrate tests.

Accuracy of the methods was determined by comparing relative densities of pure cultures of *E. coli* on the test medium with counts on a noninhibitory reference medium (PCBA) (25). Pure cultures were grown in Trypticase soy broth (BBL Microbiology Systems, Cockeysville, Md.) for 18 to 20 h at 35°C and subcultured once. After incubation, serial 10-fold dilutions were prepared in sterile 0.1% peptone water. Three replicate samples of 2 appropriate dilutions were prepared in sterile 0.1% peptone water. Three replicate samples of 2 appropriate dilutions were filtered onto each selective medium. Counts of each medium were compared with the true density obtained by spreading, with a sterile glass rod, 0.2 ml of appropriate dilutions on plate count broth (Difco) with 1.5% (wt/vol) agar (PCBA) and incubation at 35°C for 24 h (25). Specificity was evaluated by the identification of a representative number of positive (presumptive target) and negative (presumptive nontarget) colonies. All colonies were subcultured on nutrient agar (Difco) from plates with counts ranging from 10 to 100 CFU, and colony color was recorded. Comparability of the four methods was determined by analyzing 3 dilutions of water samples taken from different sources. The presumptive target and nontarget colonies were recorded from plates containing 10 to 100 colonies.

Data analysis. The data were analyzed using prepared programs for MacIntosh, Apple II, and IBM 4380 computers and the Prophet system (National Institutes of Health, Bethesda, Md.). Factorial analyses of variance were used to

TABLE 1. Comparison of recovery efficiencies

Organism	% Accuracy[a]			
	MMB	m-FC	m-FC2	mTEC
E. coli 104				
Trial 1	76	113	143	143
Trial 2	46	64	73	51
Trial 3	114	116	133	97
E. coli B	32	45	71	81
Mean	67	84	105	93

[a] Expressed as [(mean number of colonies on test medium)/(mean number of colonies on PCBA)] × 100 (n = 5).

test for differences between sites and species. Multiple correlation and regression analyses were used to determine relationships between parameters measured. Heteroscedastic data were made more homoscedastic by using the appropriate transformation before analysis. Any probability less than or equal to 0.05 was considered significant (29).

RESULTS AND DISCUSSION

Comparison of recovery efficiencies for the four test media against the nonselective reference medium showed that m-FC2 gave the best overall mean recovery (Table 1). The mTEC and m-FC methods were also relatively accurate; MMB, on the other hand, gave much lower and more variable recoveries than any of the other methods. Other studies have demonstrated accuracies for these methods that were not significantly different from ours (25). However, unlike the temperate water studies of Pagel et al. (25), we did not run accuracy trials on fecal coliforms that had been temperature stressed, i.e., 10°C for 48 h in 0.1% peptone water. The natural waters in Puerto Rico never get below 18°C, so low temperature stressing is unnecessary.

Specificity and selectivity for both freshwater and marine samples are shown in Table 2. If we accept 15% as the upper limit for a false-positive rate as Pagel et al. did (25), then none of the methods are acceptable. All the methods, except m-FC2, had higher rates of false-positive error for marine samples. However, even the freshwater samples for m-FC, m-FC2, MMB, and mTEC were grossly unacceptable, i.e., >20%. Pagel et al. (25) found that temperate freshwater false-positive errors were never greater than 18% for any method. Thus, it would seem that in tropical waters, there are more bacteria that are not *E. coli* but give a positive fecal coliform reaction for these methods of enumeration. Since the ambient water temperature is much higher and never falls below 18°C, more thermotolerant species of bacteria should be expected as background flora.

Pagel et al. (25) used an accepted limit of 5% for undetected-target error. Thus, the mTEC method had an unacceptable undetected-target error for both freshwater and marine samples. All methods, except m-FC2, had an unacceptable undetected-target error for both marine and freshwater samples. The m-FC2 method for freshwater samples had acceptable errors (<1%). All methods, except mTEC, had a much greater undetected-target error for marine samples. Temperate freshwater samples had acceptable undetected-target errors for all four methods (25). Selectivity for tropical fresh waters ranged from 69 to 84% for freshwater samples, whereas marine water selectivities were significantly lower for all methods (58 to 81%). The m-FC2 method gave the highest selectivity, as also observed by Pagel et al. (25) for temperate waters. The lowest selectivity reported for temperate water samples by these methods was 85%; thus, all of the methods had 6 to 35% lower selectivities in tropical waters (25).

Estimated densities of fecal coliforms at the different sites were high and extremely variable (Table 3). Differences of 2 to 3 orders of magnitude in recoveries were observed among the different methods for the same site. The MPN method gave significantly lower density estimates for all sites, except those associated with a coral reef. High densities of fecal coliforms have been observed for a large number of sites in Puerto Rico, many times in the complete absence of any known fecal contamination (3, 6, 9, 13, 14, 16, 21, 22, 27). Several studies by our laboratory have shown that *E. coli* can survive in tropical fresh waters in membrane diffusion chambers for weeks without any apparent loss of activity (6, 13, 15, 21). The survival of high densities of *E. coli* was even observed in tropical marine waters receiving high concentrations of organic effluents (3, 9, 13, 15, 22, 27). Other tropical environments, both marine and freshwater, have also been observed to have high densities of coliforms; however, this has always been assumed to represent gross fecal contamination, even when no source was apparent (8, 10, 12, 20).

Confirmation of target and nontarget colonies as *E. coli* showed that a maximum of 70% of the colonies was actually *E. coli* for any method (Table 4). Temperate samples typically have confirmation rates at least 18% higher for any of the four methods (25). Nontarget confirmation was low for tropical waters and not significantly different from temperate-water studies (25). Stressed or injured *E. coli* are known to give false-negative reactions (2, 4, 5, 10, 11, 17, 19, 23, 24, 26, 28); thus, tropical fresh waters seem to exert very little injury on allochthonous *E. coli* or autochthonous *E. coli* do give typical fecal coliform reactions. For all methods, 60% of the false-positive target isolates were *Klebsiella* spp., whereas *Enterobacter* spp. consisted of another 13%, and *Aeromonas* and *Pseudomonas* spp. made up another 10.4%

TABLE 2. Verification of presumptive target and nontarget colonies

Method	No. of colonies								Specificity index (%)				Selectivity index (%)	
	Presumptive target		Verified as fecal coliforms		Presumptive nontarget		Verified as nonfecal coliforms		False-positive error		Undetected-target error			
	F[a]	M[a]	F	M	F	M	F	M	F	M	F	M	F	M
MMB	24	37	17	20	8	26	7	12	29	46	6	41	75	58
m-FC	30	27	24	20	10	12	8	4	20	39	8	28	75	69
m-FC2	25	63	20	51	4	15	4	7	20	19	0	21	84	81
mTEC	22	56	15	35	10	30	6	21	32	41	21	20	69	66

[a] Abbreviations: F, fresh water; M, marine water.

TABLE 3. Densities of fecal coliforms for freshwater and marine sources

Sample source	No. of samples	Mean density (CFU/100 ml)				
		MMB	m-FC	m-FC2	mTEC	MPN
Freshwater						
River	4	1,810	2,070	3,613	453	≥240
River (recreational)	5	2,000	125	10,530	180	≥140
River + sewage	6	31,500	77,520	190,720	85,750	≥2,400
Estuarine	6	940	42,452	75,768	23,500	≥635
Marine						
Ocean + distillery	10	857	11,356	9,396	1,893	1,406
Ocean (recreational)	15	180	7,265	2,113	148	279
Harbor + sewage	5	5,250	2,475	107,103	66,050	2,400
Tuna cannery	5	27,750	10,035	15,910	6,013	2,133
Lagoon + sewage	5	1,012	13,005	38,397	28,500	1,338
Coral reef	5	105	10	10	10	1,600
Sea grass + sewage	5	10	35	123	520	809

TABLE 4. Verification of confirmed target and nontarget colonies as *E. coli*

Confirmation	% Identified as *E. coli* by indicated method			
	MMB	m-FC	m-FC2	mTEC
Target	70	60	70	60
Nontarget	15	13	23	11

(data not shown). Previous studies by our lab have shown that *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, and *Aeromonas hydrophila* also have extended survival times and high natural densities in both fresh and marine tropical waters (3, 6, 9, 13, 15, 16, 21, 22, 27). Pagel et al. (25) observed higher numbers of nontarget isolates of *E. coli* for MMB and very similar proportions for the other three methods for temperate water samples; however, this is to be expected in colder temperate waters which might select for nonthermotolerant strains of *E. coli* or cause greater thermal injury.

Comparing recoveries of fecal coliforms from various freshwater and marine sources (Table 5), the m-FC2 method with the resuscitation step gave the highest overall target colony recovery per filter for both freshwater and marine samples. The mTEC method performed better in fresh waters contaminated with sewage, whereas the marine waters gave more variable results for uncontaminated and sewage-contaminated waters. The temperate waters sampled by Pagel et al. (25) showed that mTEC and m-FC2 gave high recoveries, whereas in our study, the mTEC method gave the lowest recoveries of target colonies in tropical fresh waters and was ranked third for tropical marine waters.

Nontarget recoveries were lowest overall for the m-FC2 method for both fresh and marine samples (Table 6). The mTEC method gave significantly lower recoveries in fresh waters contaminated with sewage. Consistently lower nontarget recoveries were observed for both the m-FC2 and mTEC methods. Pagel et al. (25) found that the MMB method gave the lowest background counts in temperate fresh waters; however, the mTEC method was always second in their study. The average background counts for our tropical water samples were twice as great as those recorded for temperate water samples (25). It is not surprising to find high densities of naturally occurring mesophilic bacteria in environments that have high average temperatures. In examining the ratios of target to nontarget colonies per filter, the m-FC2 method gave the lowest ratios for both

TABLE 5. Fecal coliform recoveries from freshwater and marine sources

Sample source	No. of samples	Avg recovery[a]				Performance[b]
		MMB	m-FC	m-FC2	mTEC	
Freshwater						
River	9	85	50	42	16	MMB>m-FC=m-FC2>mTEC
River (recreational)	13	84	12	35	28	MMB>m-FC2=mTEC>m-FC
River + sewage	11	105	160	172	205	mTEC>m-FC2=m-FC>MMB
Estuarine	13	27	69	150	27	m-FC2>m-FC>MMB=mTEC
Total	46	75	73	100	70	
Marine						
Ocean + distillery	19	45	90	48	43	m-FC=m-FC2=mTEC>MMB
Ocean (recreational)	28	5	18	19	20	mTEC=m-FC2=mFC=MMB
Harbor + sewage	9	10	59	163	110	m-FC2>mTEC>mFC>MMB
Tuna cannery	9	126	127	109	109	m-FC=MMB>m-FC2=mTEC
Lagoon + sewage	9	10	82	77	6	m-FC=m-FC2>MMB=mTEC
Coral reef	9	<1	<1	<1	<1	mTEC=m-FC2=m-FC=MMB
Sea grass + sewage	9	<1	3	10	36	mTEC>m-FC2=m-FC=MMB
Total	92	26	52	53	43	

[a] Expressed as (root mean)^2 target colonies per filter.
[b] In order from highest to lowest recovery. Symbols: >, significant differences as measured by SNK test (29); =, no significant difference.

TABLE 6. Recovery of nontarget fecal coliforms from freshwater and marine sources

Sample source	No. of samples	Avg recovery[a]				Performance[b]
		MMB	m-FC	m-FC2	mTEC	
Freshwater						
River	9	16	11	5	25	m-FC2>m-FC>MMB>mTEC
River (recreational)	12	21	14	3	23	m-FC2>m-FC>MMB = mTEC
River + sewage	9	41	46	35	26	mTEC>m-FC2>MMB = m-FC
Estuarine	12	26	34	34	29	mTEC>m-FC2>MMB = m-FC
Total	42	26	26	19	26	
Marine						
Ocean + distillery	19	14	63	16	20	MMB>mTEC = m-FC2 = m-FC
Ocean (recreational)	28	8	12	7	10	MMB = m-FC = mTEC = m-FC2
Harbor + sewage	9	36	23	3	1	mTEC = m-FC2>m-FC>MMB
Tuna cannery	9	21	37	32	36	MMB = m-FC2 = mTEC = m-FC
Lagoon + sewage	9	56	32	37	16	mTEC = MMB>m-FC2 = m-FC
Coral reef	9	47	<1	<1	1	m-FC2 = m-FC = mTEC>MMB
Sea grass + sewage	9	20	2	4	45	m-FC = m-FC2>MMB = mTEC
Total	92	23	26	13	17	

[a] Expressed as (root mean)^2 target colonies per filter.
[b] In order from highest to lowest recovery. Symbols: >, significant differences as measured by SNK test (29); =, no significant difference.

fresh and marine samples, followed by MMB for freshwater samples and mTEC for marine samples (Table 7). Samples from temperate freshwater areas have shown that the mTEC and MMB methods gave equally low ratios. Large differences in the ratios were observed between methods and between types of sampling areas, much greater than those observed for temperate waters by Pagel et al. (25).

Table 8 presents a summary of the performance parameters for our study and those values obtained by Pagel et al. (25) for temperate waters. Laboratory assays for accuracy were the same, although false-positive errors were higher as a result of larger numbers of thermotolerant bacteria in tropical waters. Undetected-target errors were the same or lower in tropical waters, which is most likely caused by greater injury or thermal selection in temperate waters. The selectivity index was significantly lower for tropical waters for all methods, which is undoubtedly caused by the poor ability of the methods to indicate *E. coli* alone in tropical waters where the background flora contains other thermotolerant species. Comparability showed that recovery of both fecal coliforms and nontarget organisms was greater for all methods in tropical waters. This is probably due to less injury of allochthonous *E. coli* in tropical waters, greater survival of *E. coli* in tropical waters, and possible autochthonous origin of *E. coli* in tropical waters. The m-FC2 method performed the best of all the methods tested in both temperate (25) and tropical waters; however, in tropical waters, the performance of all methods was grossly inferior to the methods in temperate waters (25). Indeed, the m-FC2 method, our best performing method, gave higher false-positive error, higher undetected-target error, lower selectivity, and higher recovery of nontarget organisms than MMB, the worst method for temperate waters (25).

Research in other areas indicates that the situation in Puerto Rico is analogous to that found in other tropical areas of the world (18, 20). High densities of fecal coliforms have been observed for a large number of sites in Puerto Rico, many times in the complete absence of any known fecal contamination (3, 6, 9, 13, 14, 16, 21, 22, 27). In view of these findings, the isolation of fecal coliforms from waters in

TABLE 7. Ratios of nontarget to target colonies according to sample source

Sample source	Nontarget/target ratio[a]				Performance[b]
	MMB	m-FC	m-FC2	mTEC	
Freshwater					
River	0.19	0.22	0.12	1.56	m-FC2>m-FC>MMB>mTEC
River (recreational)	0.25	1.17	0.09	0.66	m-FC2>m-FC>MMB = mTEC
River + sewage	0.39	0.29	0.20	0.13	mTEC>m-FC2>MMB = m-FC
Estuarine	0.96	0.49	0.23	1.01	mTEC>m-FC2>MMB = m-FC
Total	0.45	0.54	0.16	0.86	
Marine					
Ocean + distillery	0.36	0.77	0.20	0.27	MMB>mTEC = m-FC2 = m-FC
Ocean recreational	0.73	1.95	0.72	0.67	MMB = m-FC = mTEC = m-FC2
Harbor + sewage	3.60	0.38	0.02	0.01	mTEC = m-FC2>m-FC>MMB
Tuna cannery	0.16	0.28	0.29	0.33	MMB = m-FC2 = mTEC = m-FC
Lagoon + sewage	5.60	0.39	0.48	2.67	mTEC = MMB>m-FC2 = m-FC
Coral reef	4.60	0.00	0.00	0.03	m-FC2 = m-FC = mTEC>MMB
Sea grass + sewage	2.00	0.64	0.40	0.58	m-FC = m-FC2>MMB = mTEC
Total	1.89	0.91	0.38	0.62	

[a] Expressed as (root mean)^2 nontarget colonies/(root mean)^2 target colonies per filter.
[b] In order from highest to lowest recovery. Symbols: >, significant differences as measured by SNK test (29); =, no significant difference.

TABLE 8. Summary of performance characteristics in tropical and temperate waters[a]

Method	Accuracy (% recovery)		Specificity and selectivity (%)						Comparability[b]				Rank total		Overall rank	
			False-positive error		Undetected-target error		Selectivity index		Fecal coliform recovery		Nontarget organism recovery					
	Temp[c]	Trop[c]	Temp	Trop	Temp	Trop	Temp	Trop	Temp	Trop	Temp	Trop	Temp	Trop	Temp	Trop
MMB	59 (4)	67 (4)	11 (1)	38 (4)	4 (4)	25 (4)	88 (2)	66 (3.5)	26 (4)	41 (4)	4 (1)	30 (4)	16	23.5	2	4
m-FC	89 (3)	84 (3)	16 (3)	30 (2)	1 (1)	18 (2)	85 (4)	72 (2)	41 (3)	75 (2)	11 (3)	29 (3)	17	14	3	2
m-FC2	100 (1)	105 (1)	18 (4)	19 (1)	2 (2.5)	11 (1)	90 (1)	82 (1)	48 (1)	94 (1)	15 (4)	19 (2)	13.5	7	1.5	1
mTEC	94 (2)	93 (2)	13 (2)	36 (3)	2 (2.5)	21 (3)	86 (3)	67 (3.5)	45 (2)	73 (3)	7 (2)	18 (1)	13.5	15.5	1.5	3

[a] All data labeled Temp is from Pagel et al. (25). Numbers in parentheses are simple rank orders for that category and climate zone. All calculations were done as described in the text.
[b] Expressed as the number of colonies per filter.
[c] Abbreviations: Temp, temperate; Trop, tropical.

tropical countries may not necessarily need to be a cause for health concern. Yet tropical countries have a greater need for accurate determination of the presence of recent fecal contamination due to the greater number and diversity of waterborne diseases. This exacerbates the need for alternate indicators of fecal pollution which are more accurate than those presently in use.

Considering the high densities of fecal coliforms observed at most sites in Puerto Rico, it would seem that these density estimates are in many cases grossly overestimating the degree of recent fecal contamination. In fact, fecal contamination may be indicated when none is present. Use of fecal coliforms should be discontinued as an indicator system in tropical waters. Two possibilities are open. (i) Change the indicator system and determine which bacterial genus would most closely indicate fecal contamination in tropical waters. (ii) Directly enumerate pathogens, establishing standards based on the most environmentally resistant species.

ACKNOWLEDGMENTS

We thank Jane E. Pagel for reviewing the manuscript. We thank Laura Rivera, Andrés Calvo, Enid Elias, Francisco Fuentes, Carmen Ortiz, Maria Guerra, Emilia Medina, Carlos Aranda, Laura Valdes-Collazo, and Luz Rodríguez for their technical assistance. Gary A. Toranzos also made many helpful suggestions about the manuscript. The Puerto Rico Department of Natural Resources and the Department of Marine Sciences, University of Puerto Rico, Mayagüez, generously supplied facilities and cooperation for these studies.

This work was supported by the Water Resources Research Institute of the University of Puerto Rico at Mayagüez and Sea Grant R/LR-08-87-THA1 and in part by Public Health Service grants RR-2657 and RR-8102 from the National Institutes of Health.

LITERATURE CITED

1. **American Public Health Association.** 1985. Standard methods for the examination of water and wastewater, 16th ed. American Public Health Association, New York.
2. **Baker, R. M., F. L. Singleton, and M. A. Hood.** 1983. Effects of nutrient deprivation on *Vibrio cholerae*. Appl. Environ. Microbiol. **46**:930–940.
3. **Biamón, E. J., and T. C. Hazen.** 1983. The distribution and survival of *Aeromonas hydrophila* in tropical near-shore coastal waters receiving rum distillery effluent. Water Res. **17**:319–326.
4. **Bonde, G. J.** 1977. Bacterial indicators of water pollution. Adv. Aquat. Microbiol. **1**:273–364.
5. **Cabelli, V. J., A. P. Dufour, L. J. McCabe, and M. A. Levin.** 1983. A marine recreational water quality criterion consistent with indicator concepts and risk analysis. J. Water Pollut. Control Fed. **55**:1306–1314.
6. **Carrillo, M., E. Estrada, and T. C. Hazen.** 1985. Survival and enumeration of the fecal indicators *Bifidobacterium adolescentis* and *Escherichia coli* in a tropical rain forest watershed. Appl. Environ. Microbiol. **50**:468–476.
7. **Dufour, A. P., E. R. Strickland, and V. J. Cabelli.** 1981. Membrane filter method for enumerating *Escherichia coli*. Appl. Environ. Microbiol. **41**:1152–1158.
8. **Evison, L. M., and A. James.** 1973. A comparison of the distribution of intestinal bacteria in British and East African water sources. J. Appl. Bacteriol. **36**:109–118.
9. **Fuentes, F. A., E. J. Biamon, and T. C. Hazen.** 1983. Bacterial chemotaxis to effluent from a rum distillery in tropical near-shore coastal waters. Appl. Environ. Microbiol. **46**:1438–1441.
10. **Fujioka, R. S., H. H. Hashimoto, E. B. Siwak, and R. H. Young.** 1981. Effect of sunlight on survival of indicator bacteria in seawater. Appl. Environ. Microbiol. **41**:690–696.
11. **Grabow, W. O. K., and M. du Preez.** 1979. Comparison of *m*-Endo LES, MacConkey, and Teepol media for membrane filtration counting of total coliform bacteria in water. Appl. Environ. Microbiol. **38**:351–358.
12. **Hagler, A. N., and L. C. Mendonça-Hagler.** 1981. Yeasts from marine and estuarine waters with different levels of pollution in the state of Rio de Janeiro, Brazil. Appl. Environ. Microbiol. **41**:173–178.
13. **Hazen, T. C., and C. F. Aranda.** 1981. The relationship between the distribution and abundance of bacteria and the water quality in the Rio Mameyes watershed, p. 87–111. *In* Proceedings of the Seventh Annual Puerto Rico Natural Resources Symposium. Department of Natural Resources of the Commonwealth of Puerto Rico, San Juan.
14. **Hazen, T. C., and G. W. Esch.** 1983. Effect of effluent from a nitrogen fertilizer factory and a pulp mill on the distribution and abundance of *Aeromonas hydrophila* in Albemarle Sound, North Carolina. Appl. Environ. Microbiol. **45**:31–42.
15. **Hazen, T. C., L. Prieto, A. López, and E. Biamón.** 1982. Survival and activity of fecal coliform bacteria in near-shore coastal waters, p. 128–161. *In* Proceedings of the Eighth Annual Puerto Rico Natural Resources Symposium. Department of Natural Resources of the Commonwealth of Puerto Rico, San Juan.
16. **Hazen, T. C., J. Santiago-Mercado, G. A. Toranzos, and M. Bermúdez.** 1987. What do water fecal coliforms indicate in Puerto Rico? (A review.) Bull. P.R. Med. Assoc. **79**:189–193.
17. **Hood, M. A., and G. E. Ness.** 1982. Survival of *Vibrio cholerae* and *Escherichia coli* in estuarine waters and sediments. Appl. Environ. Microbiol. **43**:578–584.
18. **Jiwa, S. F. H., K. Kikrovacek, and T. Wadström.** 1981. Enterotoxigenic bacteria in food and water from an Ethiopian community. Appl. Environ. Microbiol. **41**:1010–1019.
19. **Jones, K. L., and M. E. Rhodes-Roberts.** 1981. The survival of marine bacteria under starvation conditions. J. Appl. Bacteriol. **50**:247–258.
20. **Lavoie, M. C.** 1983. Identification of strain isolates as total and fecal coliforms and comparison of both groups as indicators of fecal pollution in tropical climates. Can. J. Microbiol. **29**:689–693.

21. **López-Torres, A. J., T. C. Hazen, and G. A. Toranzos.** 1987. Distribution and in situ survival and activity of *Klebsiella pneumoniae* in a tropical rain forest watershed. Curr. Microbiol. **15**:213–218.
22. **López-Torres, A. J., L. Prieto, and T. C. Hazen.** 1987. Comparison of the in situ survival and activity of *Klebsiella pneumoniae* and *Escherichia coli* in tropical marine environments. Microb. Ecol. **15**:1–16.
23. **McCambridge, J., and T. A. McMeekin.** 1981. Effect of solar radiation and predacious microorganisms on survival of fecal and other bacteria. Appl. Environ. Microbiol. **41**:1083–1087.
24. **McFeters, G. A., and D. G. Stuart.** 1972. Survival of coliform bacteria in natural waters: field and laboratory studies with membrane-filter chambers. Appl. Microbiol. **24**:805–811.
25. **Pagel, J. E., A. A. Qureshi, D. M. Young, and L. T. Vlassoff.** 1982. Comparison of four membrane filter methods for fecal coliform enumeration. Appl. Environ. Microbiol. **43**:787–793.
26. **Sjogren, R. E., and M. J. Gibson.** 1981. Bacterial survival in a dilute environment. Appl. Environ. Microbiol. **41**:1331–1336.
27. **Valdes-Collazo, L., A. J. Schultz, and T. C. Hazen.** 1987. Survival of *Candida albicans* in tropical marine and fresh waters. Appl. Environ. Microbiol. **53**:1762–1767.
28. **Xu, H.-S., N. Roberts, F. L. Singleton, R. W. Attwell, D. J. Grimes, and R. R. Colwell.** 1982. Survival and viability of nonculturable *Escherichia coli* and *Vibrio cholerae* in the estuarine and marine environment. Microb. Ecol. **8**:313–323.
29. **Zar, J. H.** 1984. Biostatistical analysis. Prentice-Hall, Inc., Englewood Cliffs, N.J.